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Varicella-Zoster Virus-Derived Major Histocompatibility Complex Class I-Restricted Peptide Affinity Is a Determining Factor in the HLA Risk Profile for the Development of Postherpetic Neuralgia

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ABSTRACT

Postherpetic neuralgia (PHN) is the most common complication of herpes zoster and is typified by a lingering pain that can last months or years after the characteristic herpes zoster rash disappears. It is well known that there are risk factors for the development of PHN, such as its association with certain HLA alleles. In this study, previous HLA genotyping results were collected and subjected to a meta-analysis with increased statistical power. This work shows that the alleles HLA-A*33 and HLA-B*44 are significantly enriched in PHN patients, while HLA-A*02 and HLA-B*40 are significantly depleted. Prediction of the varicella-zoster virus (VZV) peptide affinity for these four HLA variants by using one in-house-developed and two existing state-of-the-art major histocompatibility complex (MHC) class I ligand prediction methods reveals that there is a great difference in their absolute and relative peptide binding repertoires. It was observed that HLA-A*02 displays a high affinity for an ~7-fold-higher number of VZV peptides than HLA-B*44. Furthermore, after correction for HLA allele-specific limitations, the relative affinity of HLA-A*33 and HLA-B*44 for VZV peptides was found to be significantly lower than those of HLA-A*02 and HLA-B*40. In addition, HLA peptide affinity calculations indicate strong trends for VZV to avoid high-affinity peptides in some of its proteins, independent of the studied HLA allele.

IMPORTANCE

Varicella-zoster virus can cause two distinct diseases: chickenpox (varicella) and shingles (herpes zoster). Varicella is a common disease in young children, while herpes zoster is more frequent in older individuals. A common complication of herpes zoster is postherpetic neuralgia, a persistent and debilitating pain that can remain months up to years after the resolution of the rash. In this study, we show that the relative affinity of HLA variants associated with higher postherpetic neuralgia risk for varicella-zoster virus peptides is lower than that of variants with a lower risk. These results provide new insight into the development of postherpetic neuralgia and strongly support the hypothesis that one of its possible underlying causes is a suboptimal anti-VZV immune response due to weak HLA binding peptide affinity.

Primary infection by varicella-zoster virus (VZV) results in the common childhood disease varicella (chickenpox). VZV persists as a latent infection in dorsal root ganglion cells with minimal gene transcription (1). Clinical reactivation of VZV typically results in a characteristic painful dermatomal rash and is termed shingles or herpes zoster (HZ). Furthermore, HZ patients transmit VZV and thus could cause chickenpox in susceptible individuals, thereby reigniting the epidemiological VZV cycle (2, 3). It is estimated that ~10% to 30% of individuals will develop HZ in their lifetimes, with the frequency increasing to 50% for those >85 years of age (4). For many of these patients, the affected region remains painful for months or, exceptionally, years after the HZ rash resolves. If such pain persists for >3 months, this complication is called postherpetic neuralgia (PHN). Due to both its painfulness and its duration, PHN has a large impact on the health-related quality of life of HZ patients (5). PHN represents a significant proportion of HZ-related morbidity (6) and costs (7). Certain risk factors for the incidence and severity of HZ and subsequent PHN have been identified, such as VZV-specific immunity (8) and old age (6, 9, 10). However, PHN also occurs in otherwise healthy individuals. A live-attenuated VZV vaccine was shown to increase VZV-specific immunity (8) and to reduce the occurrence of HZ by ~50% and of PHN by ~65% in adults ≥60 years of age (11).

In addition, there are likely several genetic (12) and/or racial (13, 14) factors involved. Several HLA allele genotyping analyses have shown that there is a tendency for certain alleles to occur more or less frequently with PHN (15–18). Major histocompati-

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bility complex (MHC) class I molecules (encoded by the HLA genes in humans) bind short nonameric peptides derived from intracellular antigens with variable affinities for subsequent presentation to T cells. The recognition of a viral peptide on an MHC class I molecule by a $CD8^+$ cytotoxic T lymphocyte (CTL) is a necessary step in its activation to clear virus-infected host cells. Only a small fraction of peptides are able to bind to the MHC class I molecule with sufficient affinity to be presented to $CD8^+$ CTLs, and thus, this represents a critical limitation by which viral peptides are able to induce a CTL-mediated immune response (19). A large number of computational models have been developed to predict the affinity of a given peptide for a given MHC molecule with high accuracy (20–22). Such models have been successfully used to demonstrate a link between the disease severity or complications and specific affinities of HLA for peptides in other studies unrelated to herpesviruses (23, 24).

In order to better understand PHN pathogenesis and VZV vaccine target peptides, we collected data from several HLA typing studies and performed a meta-analysis to reevaluate the statistical relevance of the allele associations. We then attempt to explain these associations through differences found in the binding preferences of these HLA alleles, as predicted by MHC class I affinity models.

MATERIALS AND METHODS

Meta-analysis. The HLA frequencies for healthy and HZ-afflicted patients were extracted from three relevant reports (15, 17, 18). As the level of detail in HLA typing differed between the different studies, we chose to work at the level of HLA allele groups. Individuals described as having no history of HZ defined the control group. The PHN-negative (PHN^-) group was made up of patients with HZ who did not develop PHN, while the PHN-positive (PHN^+) group consisted of HZ patients who still experienced persistent pain at least 3 months after the rash resolved. These three groups were not present in all three studies, and thus, the tested alleles differ between the control-PHN^+ and PHN^+-PHN^- group comparisons. The counts were combined, and a hypergeometric distribution was used to estimate the enrichment *P* values for the HLA alleles in specific groups. The *P* value cutoff used is 0.05, with a Bonferroni correction for multiple testing, in this case the number of alleles tested.

HLA predictors and classifiers. There are many methods available to predict the affinity of MHC class I proteins and their ligand peptides. However, for the purposes of this article, we required that the method contained a model for the four HLA alleles to be studied (HLA-A*02, HLA-A*33, HLA-B*40, and HLA-B*44). If a method allowed a choice of the specific HLA protein sequence, we chose the most common HLA protein from the meta-analysis unless specified otherwise, i.e., HLA-A*02:01, HLA-A*33:03, HLA-B*40:06, and HLA-B*44:03. Furthermore, the method had to be able to screen the entire VZV genome and not just a single protein or peptide. We use three complementary methods that match these criteria, namely, NetCTLpan, the stabilized matrix method (SMM), and an in-house-developed approach called CRFMHC. Both NetCTLpan and SMM give an exact prediction of the affinity value for a given peptide. NetCTLpan is based on artificial neural networks and has a public Web version (25). SMM predicts the 50% inhibitory concentration (IC_{50}) values for each peptide, and a stand-alone version of this tool is provided through the Immune Epitope Database (IEDB) (26). For SMM, the HLA-A*33:01 and HLA-B*40:01 models were used. CRFMHC is a repurposed phosphorylation predictor (27) retrained on the IEDB data (28). Data for HLA-A*33:01 and HLA-B*40:01 were used for training, as the other HLA protein sequences had insufficient data in the IEDB. This method is based on conditional random fields (CRFs), so it can give only a conditional probability of peptide binding and not the absolute affinity. Therefore, it is used only for relative affinity calculations. See below for a detailed description of the CRFMHC method.

Description of the CRFMHC method. CRFs were originally designed for sequence labeling problems in natural language processing (29). For such problems, the goal is to predict a sequence of labels for a given sequence of observations. Unlike other probabilistic models, such as the hidden Markov models from which they are derived, CRFs do not need to model the joint probability between observations and labels; that is, they do not need to account for any dependencies between observations. For this reason, they are highly useful when the dependencies between observations are extremely complex. As such, they have also been used with great success within the field of bioinformatics, e.g., for the prediction of phosphorylation sites (27) and regulatory binding sites (30). More information on the algorithmic and statistical support of CRFs can be found in the previous reports mentioned above.

Interestingly, the prediction of MHC binding peptides is very similar to the prediction of phosphorylation sites. The model presented previously by Dang et al. calculates a conditional probability that a given sequence of 9 amino acids is phosphorylated by a given kinase (27). This model was first trained on a set of known phosphorylated sequences before it was applied to the classification of sequences with an unknown phosphorylation status. This procedure can be readily translated to the prediction of MHC class I affinity. Here the model calculates a conditional probability that a given sequence of 9 amino acids is bound to a given MHC allele. This model can also be first trained on a set of sequences known to be bound or not bound by the given MHC allele. CRFMHC is thus exactly the same model as that described previously by Dang et al. (27), except that a negative set is explicitly included in model training, and thus, the probability score is used as is, without the need for inequality correction. The training data for these models were derived from the IEDB (28).

Absolute affinity prediction. The protein sequences of all VZV peptides were extracted from the NCBI database (GenBank accession number NC_001348). A fasta file containing these VZV proteins was run through NetCTLpan, and each 9-mer peptide was extracted, which corresponds to 34,947 unique peptides. The reported MHC affinity scores were used in this analysis. No ensemble method can be used for absolute affinity predictions, as every method uses a different metric for peptide affinity, and any combination would result in a loss of biological meaning of the score. Depletion *P* values for high-affinity peptides in VZV proteins were estimated based on a hypergeometric distribution given the number of possible peptides from a particular protein and the observed amount of high-affinity peptides found in the protein and in the entire proteome.

Relative affinity prediction. The relative affinity of each allele was determined by comparison to the affinity for highly expressed human proteins. This list consists of 107 human proteins extracted from PaxDb that were most highly expressed (31) (see Table S1 in the supplemental material). The proteins included consisted mostly of ribosomal proteins and other housekeeping proteins. The three MHC prediction methods were applied to the VZV genome and the list of human proteins. The highest- and lowest-scoring human peptides were used to normalize the affinity values for each method and each allele separately. After this step, a single affinity score was calculated by taking the mean of the results from the three methods. The normalization step against the human peptides was then repeated with the combined score. This results in an affinity scoring system where values of >1 signify peptides that have a higher affinity than the best-binding human peptide and values of <0 have a lower affinity than the worst-binding human peptide.

RESULTS

Meta-analysis of HLA genotyping reveals PHN-associated alleles. The HLA frequencies from different genotyping studies were combined in a single data set, and the enrichments for the different patient groups (control, PHN^+, and PHN^-) were recalculated (15–18). The comparison between the PHN^+ patients and

TABLE 1 HLA frequencies and enrichments in patients with PHN compared to the control group

Allele	Frequency (%) for group		*P* value[a]	
	PHN$^+$	Control	Enrichment	Depletion
HLA-A*01	0.4	1.0	0.930	0.288
HLA-A*02	13.6	24.9	1.000	5.31×10^{-5}*
HLA-A*03	0.4	0.1	0.445	0.935
HLA-A*11	6.4	9.7	0.962	0.066
HLA-A*24	37.4	36.1	0.381	0.673
HLA-A*26	10.2	11.0	0.674	0.414
HLA-A*30	0.0	0.1	1.000	0.745
HLA-A*31	10.2	10.3	0.563	0.531
HLA-A*33	21.5	6.8	2.76×10^{-10}*	1.000
HLA-B*07	5.3	6.5	0.805	0.287
HLA-B*13	1.4	1.1	0.467	0.755
HLA-B*15	8.5	6.5	0.164	0.893
HLA-B*17	0.4	0.1	0.460	0.930
HLA-B*27	0.0	0.6	1.000	0.213
HLA-B*35	6.7	8.0	0.800	0.283
HLA-B*37	0.4	0.8	0.885	0.407
HLA-B*38	0.0	0.1	1.000	0.735
HLA-B*39	3.5	3.7	0.613	0.534
HLA-B*40	11.3	11.9	0.635	0.449
HLA-B*44	20.5	6.5	3.19×10^{-10}*	1.000
HLA-B*46	3.2	6.3	0.986	0.031
HLA-B*48	1.8	2.9	0.903	0.205
HLA-B*51	7.4	7.8	0.621	0.481
HLA-B*52	10.6	11.4	0.671	0.413
HLA-B*54	7.8	8.0	0.599	0.502
HLA-B*55	1.8	3.8	0.975	0.065
HLA-B*56	0.4	1.4	0.976	0.131
HLA-B*58	0.4	0.1	0.460	0.930
HLA-B*59	1.4	2.0	0.820	0.353
HLA-B*60	1.1	1.8	0.870	0.300
HLA-B*61	1.4	3.6	0.985	0.046
HLA-B*62	2.5	3.2	0.787	0.354
HLA-B*67	2.1	1.0	0.139	0.950
HLA-B*70	0.4	0.4	0.709	0.713
HLA-B*75	0.0	0.4	1.000	0.396
HLA-DRB1*01	7.4	7.1	0.475	0.631
HLA-DRB1*04	17.0	17.5	0.600	0.474
HLA-DRB1*08	10.7	10.1	0.432	0.657
HLA-DRB1*09	12.6	17.0	0.965	0.054
HLA-DRB1*10	0.0	0.1	1.000	0.735
HLA-DRB1*11	3.3	2.4	0.269	0.850
HLA-DRB1*12	5.6	3.6	0.116	0.937
HLA-DRB1*13	17.0	5.6	5.99×10^{-8}*	1.000
HLA-DRB1*14	6.7	6.9	0.609	0.502
HLA-DRB1*15	12.2	14.8	0.876	0.172
HLA-DRB1*16	1.1	0.9	0.519	0.741

[a] Asterisks indicate a significant value according to a *P* value cutoff of 1×10^{-3}.

TABLE 2 HLA frequencies and enrichments in herpes zoster patients with PHN and those without PHN

Allele	Frequency (%) for group		*P* value[a]	
	PHN$^+$	PHN$^-$	Enrichment	Depletion
HLA-A*01	0.4	0.0	0.545	1.000
HLA-A*02	13.6	22.2	0.995	0.009
HLA-A*03	0.4	0.0	0.545	1.000
HLA-A*11	6.4	6.8	0.638	0.506
HLA-A*24	37.4	43.4	0.927	0.102
HLA-A*26	10.2	10.4	0.592	0.527
HLA-A*31	10.2	9.5	0.462	0.656
HLA-A*33	21.5	7.7	1.33×10^{-5}*	1.000
HLA-B*07	5.3	7.0	0.834	0.277
HLA-B*13	1.4	0.5	0.283	0.942
HLA-B*15	8.5	8.4	0.550	0.579
HLA-B*17	0.4	0.0	0.568	1.000
HLA-B*35	6.7	7.0	0.618	0.523
HLA-B*37	0.4	0.5	0.814	0.678
HLA-B*39	3.5	4.2	0.731	0.440
HLA-B*40	11.3	22.3	1.000	7.35×10^{-4}*
HLA-B*44	20.5	6.5	4.89×10^{-6}*	1.000
HLA-B*46	3.2	5.6	0.938	0.137
HLA-B*48	1.8	4.2	0.970	0.090
HLA-B*51	7.4	7.0	0.497	0.640
HLA-B*52	10.6	13.5	0.870	0.198
HLA-B*54	7.8	7.4	0.515	0.619
HLA-B*55	1.8	2.3	0.778	0.448
HLA-B*56	0.4	0.9	0.920	0.398
HLA-B*58	0.4	0.9	0.920	0.398
HLA-B*59	1.4	0.9	0.478	0.814
HLA-B*60	1.1	0.0	0.183	1.000
HLA-B*61	1.4	0.0	0.103	1.000
HLA-B*62	2.5	0.0	0.019	1.000
HLA-B*67	2.1	0.9	0.251	0.925
HLA-B*70	0.4	0.0	0.568	1.000
HLA-DRB1*01	7.4	5.9	0.318	0.799
HLA-DRB1*03	0.0	0.5	1.000	0.449
HLA-DRB1*04	17.0	20.5	0.861	0.197
HLA-DRB1*08	10.7	15.9	0.966	0.060
HLA-DRB1*09	12.6	18.6	0.976	0.043
HLA-DRB1*10	0.0	0.5	1.000	0.449
HLA-DRB1*11	3.3	1.4	0.133	0.959
HLA-DRB1*12	5.6	2.3	0.053	0.983
HLA-DRB1*13	17.0	8.2	0.003	0.999
HLA-DRB1*14	6.7	5.0	0.281	0.834
HLA-DRB1*15	12.2	20.9	0.997	0.007
HLA-DRB1*16	1.1	0.5	0.392	0.909

[a] Asterisks indicate a significant value according to a *P* value cutoff of 1.1×10^{-3}.

the control group is presented in Table 1. The found associations are similar to those reported in the individual studies but are confirmed with stronger statistical power. Patients with PHN have a significantly higher tendency to have the alleles HLA-A*33, HLA-B*44, and HLA-DRB1*13 than healthy control patients. In addition, the HLA-A*02 allele is significantly enriched in the control group, indicating that this allele somehow reduces the risk for PHN or HZ. This HLA-A*02 tendency was reported in only one of the studies included in our meta-analysis. Very similar results can be found by comparing the HLA frequencies between HZ patients with and those without PHN (Table 2). HLA-A*33 and HLA-B*44 remain enriched in the PHN$^+$ group, indicating that this association is, at least in part, specific to PHN and not just to HZ. In addition, HLA-B*40 is now significantly depleted in PHN$^+$ patients, suggesting that this allele might also reduce the relative risk for PHN. The enrichment and depletion *P* values for HLA-DRB1*13 and HLA-A*02, respectively, are also low in this comparison but no longer pass the cutoff after stringent Bonferroni multiple-testing correction. Thus, HLA-DRB1*13 and HLA-A*02 might have an effect mainly as HZ-associated factors. In the analyses described below, we focused on the MHC class I alleles, as

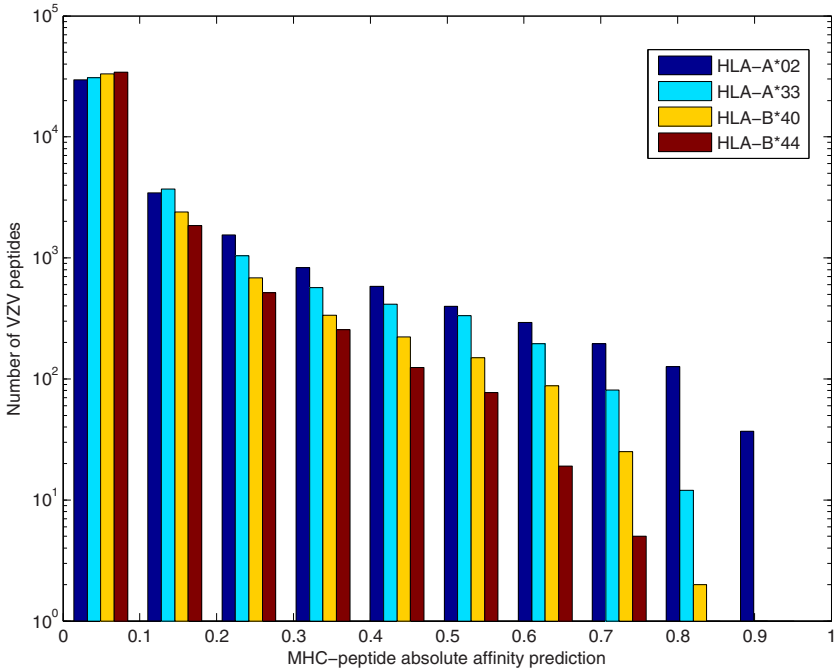

FIG 1 Bar plot of the affinity values of HLA-A*02, HLA-A*33, HLA-B*40, and HLA-B*44 for the VZV peptides, as predicted by NetCTLpan. Higher values on the *x* axis signify high affinity. The *y* axis provides the number of VZV peptides found within the affinity range.

only a single class II allele, i.e., HLA-DRB1*13, was found to have a significant impact on the incidence of PHN in a single comparison.

HLA-B*44 has a limited repertoire of high-affinity VZV peptides. A likely hypothesis is that the association between specific HLA alleles and PHN stems from the affinity at which the VZV-derived peptides are being presented by the MHC I molecules to $CD8^+$ T cells. Indeed, one of the key differences between the different HLA groups is their specificity and affinity for intracellularly processed peptides derived from viral proteins. The affinity of the potential immunogenic peptides derived from the VZV genome and the HLA allelic variants that can be associated with a higher PHN incidence (HLA-A*33 and HLA-B*44) or with a lower incidence (HLA-A*02 and HLA-B*40) can be computationally predicted. This prediction is based on computational models that are specifically trained for each HLA allele and that consider specific features (e.g., anchor residues) in the amino acid sequence of the peptide. For such a procedure, the NetCTLpan method is most commonly considered the current state of the art (25). Prediction of the peptide affinity of the entire VZV proteome for each of the four above-described HLA allelic variants reveals a distribution of many low-affinity and few high-affinity binders, as shown in Fig. 1. While the distributions of the low-affinity peptides for each HLA variant are mostly similar, the high-affinity distributions show great differences. From these results, it is clear that HLA-A*02, with a low PHN risk, has the highest affinity for several VZV peptides and that HLA-B*44, with a high PHN risk, has the lowest affinity. The difference between the affinity distributions for HLA-A*02 and HLA-B*44 can be quantified by a two-sample Kolmogorov-Smirnov statistic, which estimates a *P* value of 3.75×10^{-141}. At a cutoff value of 0.4, a reasonable threshold for NetCTLpan, there is a 7-fold-higher number of high-affinity VZV peptides for HLA-A*02 than for HLA-B*44 (1,531 and 202, respectively).

HLA-A*33 and HLA-B*44 show a low relative affinity for VZV peptides. The absolute affinity values do not present a complete picture of HLA preference for VZV peptides. Simply from this analysis, it is not known if the difference in peptide affinity is due to VZV amino acid sequences or just a characteristic of the HLA molecule itself. Some HLA alleles might be assigned lower affinity scores due simply to computational limits of the models or biological limits of the strength of peptide binding for specific MHC sequence configurations. Indeed, recent work has shown that some HLA alleles, including HLA-B*44, have a smaller peptide repertoire (32). A more proper test would be to compare the affinity values for each allele to a common reference prior to comparison among alleles. As a reference, we used a collection of highly expressed human proteins. This reference has biological meaning in the sense that these proteins are the most likely competitors for HLA binding with any viral proteins that may be produced in the cell. For any viral peptide to be frequently presented on the HLA molecule, it must have a sufficiently high affinity compared to human peptides. In order to reduce the false-positive rate, the results from three different complementary methods were combined for more reliable predictions. This analysis revealed a single VZV peptide that consistently scored higher than any human peptide for HLA-A*02 over all three methods. This peptide was ILIEGIFFV, derived from ribonucleotide reductase subunit 2 (open reading frame 18 [ORF18]) and was previously identified as a strong immunogenic peptide in HLA-A*02:01-positive patients with a history of VZV infection (33). The combined score revealed a single peptide with a higher score than any human peptide for HLA-A*33, namely, HFFLH VCFR from UL32 (ORF26). In the case of HLA-B*40, three

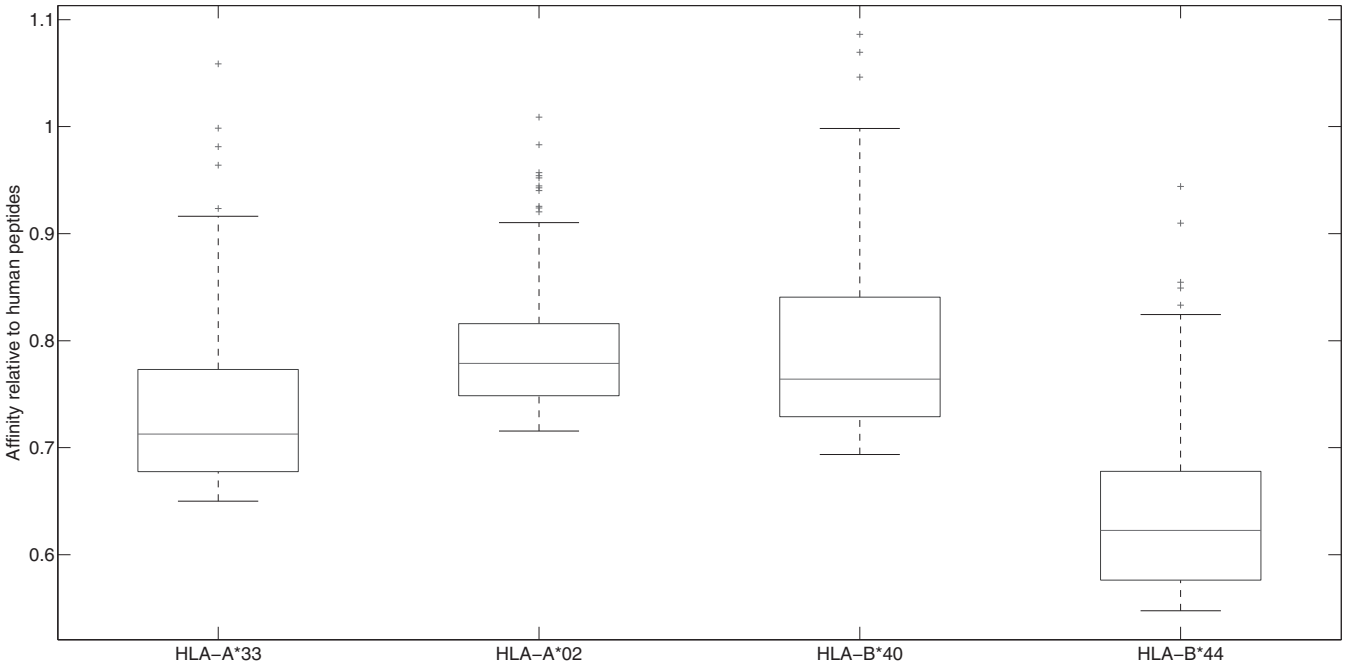

FIG 2 Box plots of the distribution of relative affinities of HLA-A*02, HLA-A*33, HLA-B*40, and HLA-B*44 for the top 200 peptides from a combination of three complementary prediction methods. A value of 1 on the *y* axis indicates the highest-affinity human peptide for a given HLA allele.

VZV peptides had a higher combined score than any human peptide, namely, REYCVAPPV (ORF16), AEICTRLGL (ORF29), and GEFGNILPL (ORF36). No such peptide was found for HLA-B*44. Indeed, even prior to combining the scores of the two methods, namely, NetCTLpan and CRFMHC, we did not find any VZV peptides with a normalized score of >1 for HLA-B*44. However, as HLA molecules will likely still bind VZV peptides even if they have a lower relative score than the best-scoring human peptide, we analyzed the scores of peptides under this threshold. As shown in Fig. 2, the relative scores of the top 200 VZV peptides for each allele differ greatly. The HLA-A*02 and HLA-B*40 scores, i.e., those of the low-PHN-risk alleles, are higher than those of HLA-A*33 and HLA-B*44, i.e., those associated with PHN. This difference is highly significant, as a two-sample Kolmogorov-Smirnov test estimates a *P* value of 3.04×10^{-26} between the scores of HLA-A*02 and HLA-A*33 and a *P* value of 2.21×10^{-64} between the scores of HLA-A*02 and HLA-B*44.

VZV IE62 is strongly depleted for high-affinity peptides across HLA alleles. A striking observation was made for the distribution of the high-affinity peptides in the VZV proteome. Using the previous absolute affinity cutoff of 0.4 for NetCTLpan, a clear pattern emerges, where some VZV proteins have a much lower fraction of high-affinity peptides than others. We can quantify this by comparing the actual number of high-affinity peptides for a protein to the number of expected high-affinity peptides if they were uniformly distributed across the genome. Assigning a *P* value to the probability of this depletion by chance for each protein and for each allele, as shown in Fig. 3, reveals that the IE62 protein (ORF62) is depleted for high-affinity peptides across all tested HLA alleles. The IE62 protein corresponds to an ICP4 transcriptional regulator known to be expressed during the immediate early stages of viral activation (34). For example, 30 high-affinity peptides in IE62 can be found in the predictions for HLA-A*02. Given the size of the protein, one would actually expect ~54 high-affinity peptides if these were distributed across the VZV proteome in a uniform manner; i.e., 4.12% of all VZV peptides exceed the cutoff value of 0.4, and IE62 has 1,302 9-mer peptides. This corresponds to a significant depletion of HLA-A*02 high-affinity peptides in this protein, with a *P* value of 1.23×10^{-7}. Similar results were found for a larger selection of HLA alleles, other affinity cutoffs, and other affinity prediction methods (see Tables S2 to S5 in the supplemental material). Interestingly, VZV IE63, another VZV immediate early protein that is found in ganglia and is implicated in VZV reactivation (35, 36), also shows depletion for several HLA alleles, including the abundant HLA-A*02 allele. Indeed, together with VZV IE62, VZV IE63 has the lowest affinity for HLA-A*02.

DISCUSSION

Our meta-analysis supports the finding made by each independent study that HLA-A*33 and HLA-B*44 are likely strong risk factors for PHN. Furthermore, this analysis revealed several new patterns, such as the depletion of HLA-A*02 in PHN patients compared to healthy controls and of HLA-B*40 in PHN patients compared to other HZ patients without PHN. We acknowledge some caveats to this analysis. First, many of the included studies focused on individuals of Japanese origin, creating a bias in the allele frequency. While this does not affect the conclusions regarding which alleles are more or less associated with PHN, it does mean that for many common alleles that are rare in the Japanese population, insufficient information is available to form a conclusion on their role in PHN. In addition, there has not been a sufficiently large HLA genotyping study of HZ patients without a focus on PHN. This means that we cannot establish with certainty whether these associations are present in all HZ patients and thus

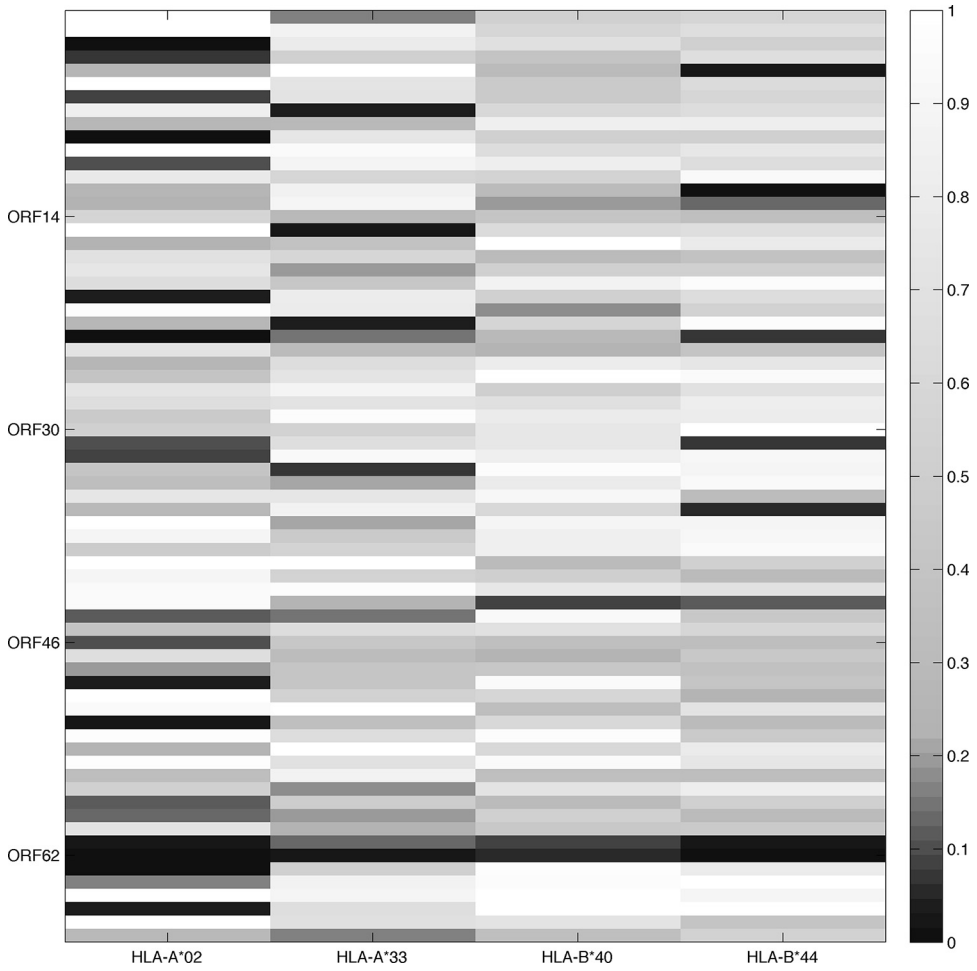

FIG 3 Heat map of the *P* values for each VZV open reading frame corresponding to the probability that one would find the same number of or fewer high-affinity peptides in a protein of a given size. The *y* axis features the VZV ORFs in numerical order, and the *x* axis shows the four HLA allelic variants of interest. The shading corresponds to the *P* value, where darker shading indicates a lower *P* value and thus a more significant depletion.

confound the associations with PHN. However, as the results of comparisons of PHN^+ patients versus healthy controls and PHN^+ versus PHN^- patients were fairly consistent, we can assume that these alleles have at least some role in PHN. The above-mentioned caveats illustrate the need for more (Western and African) studies typing HLA in HZ patients, PHN patients, and control populations.

The absolute affinity for the VZV proteome is less informative for our analysis, as it is strongly related to the limitations of the allele itself, independent of VZV. However, in this case, it was found that the HLA-B*44 variant had the lowest affinity and that HLA-A*02 had the highest affinity for VZV peptides out of all alleles tested. More relevant were the relative affinity results, which remove any intrinsic bias of the allele itself but focus on the affinity of the HLA molecule for VZV peptides in relation to human peptides. This analysis indicated that HLA-B*44 and HLA-A*33, the two alleles most enriched in PHN patients, have a poor relative affinity for VZV peptides. Concretely, the affinity prediction results indicate that VZV peptides have low competitive affinity for HLA-B*44 and HLA-A*33 in comparison to other peptides that are present in human cells. In addition, they will not bind for as long or as strongly to the HLA-B*44 variant as they do to other variants. These results contrasted with the high relative VZV peptide affinity of HLA-A*02 and HLA-B*40, which were strongly depleted in PHN patients according to our meta-analysis. If this is indeed the cause of the association of HLA-B*44 and HLA-A*33 with PHN, this would support the theory that one of the potential causes of PHN can be found in a weak or delayed $CD8^+$ response to VZV reactivation. This would also match the observation that PHN is more common in elderly or immunocompromised individuals (37, 38). The strong association of HLA*B44 with PHN is also interesting, as it is more common in individuals of Caucasian origin (39), who also have a greater predisposition to develop HZ (13). However, it should be noted that the *in silico* prediction methods used predict only the theoretical affinity of the peptide for the MHC molecule and do not reveal if this peptide is actually being processed *in vivo* and/or recognized by a CD8 T-cell receptor, a necessary step in the initiation of an immune response. While there are models that predict the immunogenicity of a presented peptide, they are still plagued by poor accuracy, and most remain limited to very common HLA alleles (40).

Another observation of interest was the significant depletion of high-affinity peptides in VZV IE62 for almost every HLA variant

tested and in VZV IE63 for HLA-A*02. These proteins are expressed during the early stage of viral reactivation, and there is evidence to support that these proteins may also be expressed during latency (35, 41). One possible hypothesis is that there is strong evolutionary pressure to introduce mutations in these proteins that remove potential high-affinity peptides. However, further research is necessary to evaluate if herpesviruses have indeed adapted their protein sequence to avoid binding to MHC class I molecules during latency and in the immediate early stage of viral activation to reduce the likelihood of a subsequent $CD8^+$ immune response, as has been observed for other viral proteomes (42–44). This would then be a system complementary to the other mechanisms available to VZV to avoid an immune response (45). However, both VZV IE62 and VZV IE63 are known to be immunogenic in individuals exposed to VZV, indicating that some MHC binding peptides are still present in the protein (36, 46, 47). Given the size of the VZV IE62 protein and its expression during a critical stage, this is not surprising. Indeed, our analysis showed that IE62 has around half of the expected number of high-affinity peptides for most HLA alleles but was not devoid of them. It may very well be that the remaining MHC binding peptides cannot be modified without compromising the function of the protein. The strong depletion of high-affinity peptides for HLA-A*02 is then also unsurprising for both VZV IE62 and IE63 in this context, as this is a very common allele (39), and it has been shown that viruses can undergo escape mutations specific for the HLA types of their host population (42–44). Within the scope of this paper, we must, however, wonder what the impact of this depletion is on the development of HZ and subsequent PHN, as the HLA variant with the most significant depletion was HLA-A*02, which was shown to protect against HZ/PHN. In line with the remainder of our results and the high frequency of HLA-A*02, an initial hypothesis is that without significant depletion against this HLA variant, protection against HZ/PHN would be even greater. The depletion for high-affinity peptides in VZV IE62 could therefore have evolved due to selective pressure over many generations of VZV human infections. However, the depletion against HLA-A*02 in this protein seems to be insufficient to evade the protection offered by this HLA variant. Interestingly, it was recently shown that VZV IE63 immune responses are very sensitive to reexposure to circulating VZV (47). Our findings thus suggest that both VZV IE63 and VZV IE62 might be reasonable candidates for future, more effective HZ vaccines through boosting of CTL-mediated immunity against these peptides.

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